

WHAT IS CLAIMED IS:

1. A method of expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells, the method comprising the steps of:

- (a) obtaining undifferentiated hemopoietic stem cells or progenitor cells; and
- (b) seeding said undifferentiated hemopoietic stem cells or progenitor cells into a stationary phase plug-flow bioreactor in which a three dimensional stromal cell culture has been pre-established on a substrate in the form of a sheet, said substrate including a non-woven fibrous matrix forming a physiologically acceptable three-dimensional network of fibers, thereby expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells.

2. The method of claim 1, wherein said undifferentiated hemopoietic stem cells or progenitor cells are cells isolated from a tissue selected from the group consisting of cord blood, mobilized peripheral blood and bone-marrow.

3. The method of claim 1, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture share common HLA antigens.

4. The method of claim 1, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from a single individual.

5. The method of claim 1, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from different individuals.

6. The method of claim 1, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from the same species.

7. The method of claim 1, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from different species.

8. The method of claim 1, wherein stromal cells of said stromal cell culture are grown to a density of at least 5×10^6 cells per a cubic centimeter of said substrate.

9. The method of claim 1, wherein stromal cells of said stromal cell culture are grown to a density of at least 10^7 cells per a cubic centimeter of said substrate.

10. The method of claim 1, wherein said step of seeding said undifferentiated hemopoietic stem cells or progenitor cells into said stationary phase plug-flow bioreactor is effected while flow in said bioreactor is shut off for at least 10 hours following said seeding.

11. The method of claim 1, wherein said fibers form a pore volume as a percentage of total volume of from 40 to 95 % and a pore size of from 10 microns to 100 microns.

12. The method of claim 1, wherein said matrix is made of fiber selected from the group consisting of flat, non-round, and hollow fibers and mixtures thereof, said fibers being of from 0.5 microns to 50 microns in diameter or width.

13. The method of claim 1, wherein said matrix is composed of ribbon formed fibers having a width of from 2 microns to 20 microns, and wherein the ratio of width to thickness of the fibers is at least 2:1.

14. The method of claim 1, wherein said matrix having a pore volume as a percentage of total volume of from 60 to 95%.

15. The method of claim 1, wherein the matrix has a height of 50-1000 μm .

16. The method of claim 1, wherein the material of the matrix is selected from the group consisting of polyesters, polyalkylenes, polyfluorochloroethylenes, polyvinyl chloride, polystyrene, polysulfones, cellulose acetate, glass fibers, and inert metal fibers.

17. The method of claim 1, wherein the matrix is in a shape selected from the group consisting of squares, rings, discs, and cruciforms.

18. The method of claim 1, wherein the matrix is in the form of a disc.

19. The method of claim 1, wherein the matrix is coated with poly-D-lysine.

20. The method of claim 1, further comprising the step of isolating said undifferentiated hemopoietic stem cells or progenitor cells.

21. A method of expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells, the method comprising the steps of:

- (a) obtaining undifferentiated hemopoietic stem cells or progenitor cells; and
- (b) culturing said undifferentiated hemopoietic stem cells or progenitor cells in a medium containing a stromal cell conditioned medium, said stromal cell conditioned medium being derived from a stationary phase plug-flow bioreactor in which a three dimensional stromal cell culture has been established on a substrate in the form of a sheet, said substrate including a non-woven fibrous matrix forming a physiologically acceptable three-dimensional network of fibers, thereby expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells.

22. The method of claim 21, wherein said undifferentiated hemopoietic stem cells or progenitor cells are cells isolated from a tissue

selected from the group consisting of cord blood, mobilized peripheral blood and bone-marrow.

23. The method of claim 21, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture share common HLA antigens.

24. The method of claim 21, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from a single individual.

25. The method of claim 21, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from different individuals.

26. The method of claim 21, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from the same species.

27. The method of claim 21, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from different species.

28. The method of claim 21, wherein stromal cells of said stromal cell culture are grown to a density of at least 5×10^6 cells per a cubic centimeter of said substrate.

29. The method of claim 21, wherein stromal cells of said stromal cell culture are grown to a density of at least 10^7 cells per a cubic centimeter of said substrate.

30. The method of claim 21, wherein said fibers form a pore volume as a percentage of total volume of from 40 to 95 % and a pore size of from 10 microns to 100 microns.

31. The method of claim 21, wherein said matrix is made of fiber selected from the group consisting of flat, non-round, and hollow fibers and mixtures thereof, said fibers being of from 0.5 microns to 50 microns in diameter or width.

32. The method of claim 21, wherein said matrix is composed of ribbon formed fibers having a width of from 2 microns to 20 microns, and wherein the ratio of width to thickness of the fibers is at least 2:1.

33. The method of claim 21, wherein said matrix having a pore volume as a percentage of total volume of from 60 to 95%.

34. The method of claim 21, wherein the matrix has a height of 50-1000 μm .

35. The method of claim 21, wherein the material of the matrix is selected from the group consisting of polyesters, polyalkylenes, polyfluorochloroethylenes, polyvinyl chloride, polystyrene, polysulfones, cellulose acetate, glass fibers, and inert metal fibers.

36. The method of claim 21, wherein the matrix is in a shape selected from the group consisting of squares, rings, discs, and cruciforms.

37. The method of claim 21, wherein the matrix is in the form of a disc.

38. The method of claim 21, wherein the matrix is coated with poly-D-lysine.

39. A method of preparing a stromal cell conditioned medium useful in expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells, the method comprising the steps of:

(a) establishing a stromal cell culture in a stationary phase plug-flow bioreactor on a substrate in the form of a sheet, said substrate including a non-woven fibrous matrix forming a physiologically acceptable three-dimensional network of fibers, thereby expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells;
and

(b) when a desired stromal cell density has been achieved, collecting medium from said stationary phase plug-flow bioreactor, thereby obtaining the stromal cell conditioned medium useful in expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells.

40. The method of claim 39, wherein stromal cells of said stromal cell culture are grown to a density of at least 5×10^6 cells per a cubic centimeter of said substrate.

41. The method of claim 39, wherein stromal cells of said stromal cell culture are grown to a density of at least 10^7 cells per a cubic centimeter of said substrate.

42. The method of claim 39, wherein said fibers form a pore volume as a percentage of total volume of from 40 to 95 % and a pore size of from 10 microns to 100 microns.

43. The method of claim 39, wherein said matrix is made of fiber selected from the group consisting of flat, non-round, and hollow fibers and mixtures thereof, said fibers being of from 0.5 microns to 50 microns in diameter or width.

44. The method of claim 39, wherein said matrix is composed of ribbon formed fibers having a width of from 2 microns to 20 microns, and wherein the ratio of width to thickness of the fibers is at least 2:1.

45. The method of claim 39, wherein said matrix having a pore volume as a percentage of total volume of from 60 to 95%.

46. The method of claim 39, wherein the matrix has a height of 50-1000 μm .

47. The method of claim 39, wherein the material of the matrix is selected from the group consisting of polyesters, polyalkylenes, polyfluorochloroethylenes, polyvinyl chloride, polystyrene, polysulfones, cellulose acetate, glass fibers, and inert metal fibers.

48. The method of claim 39, wherein the matrix is in a shape selected from the group consisting of squares, rings, discs, and cruciforms.

49. The method of claim 39, wherein the matrix is in the form of a disc.

50. The method of claim 39, wherein the matrix is coated with poly-D-lysine.

51. A method of transplanting undifferentiated hemopoietic stem cells or progenitor cells into a recipient, the method comprising the steps of:

- (a) expanding/maintaining the undifferentiated hemopoietic stem cells or progenitor cells by:
 - (i) obtaining undifferentiated hemopoietic stem cells or progenitor cells; and
 - (ii) seeding said undifferentiated hemopoietic stem cells or progenitor cells into a stationary phase plug-flow bioreactor in which a three dimensional stromal cell culture has been pre-established on a substrate in the form of a sheet, said substrate including a non-woven fibrous matrix forming a physiologically

acceptable three-dimensional network of fibers, thereby expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells; and

- (b) transplanting said undifferentiated hemopoietic stem cells or progenitor cells resulting from step (a) in the recipient.

52. The method of claim 51, wherein said undifferentiated hemopoietic stem cells or progenitor cells are cells isolated from a tissue selected from the group consisting of cord blood, mobilized peripheral blood and bone-marrow.

53. The method of claim 51, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture share common HLA antigens.

54. The method of claim 51, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from a single individual.

55. The method of claim 51, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from different individuals.

56. The method of claim 51, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from the same species.

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57. The method of claim 51, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from different species.

58. The method of claim 51, wherein stromal cells of said stromal cell culture are grown to a density of at least 5×10^6 cells per a cubic centimeter of said substrate.

59. The method of claim 51, wherein stromal cells of said stromal cell culture are grown to a density of at least 10^7 cells per a cubic centimeter of said substrate.

60. The method of claim 51, wherein said step of seeding said undifferentiated hemopoietic stem cells or progenitor cells into said stationary phase plug-flow bioreactor is effected while flow in said bioreactor is shut off for at least 10 hours following said seeding.

61. The method of claim 51, wherein said fibers form a pore volume as a percentage of total volume of from 40 to 95 % and a pore size of from 10 microns to 100 microns.

62. The method of claim 51, wherein said matrix is made of fiber selected from the group consisting of flat, non-round, and hollow fibers and mixtures thereof, said fibers being of from 0.5 microns to 50 microns in diameter or width.

63. The method of claim 51, wherein said matrix is composed

of ribbon formed fibers having a width of from 2 microns to 20 microns, and wherein the ratio of width to thickness of the fibers is at least 2:1.

64. The method of claim 51, wherein said matrix having a pore volume as a percentage of total volume of from 60 to 95%.

65. The method of claim 51, wherein the matrix has a height of 50-1000 μm .

66. The method of claim 51, wherein the material of the matrix is selected from the group consisting of polyesters, polyalkylenes, polyfluorochloroethylenes, polyvinyl chloride, polystyrene, polysulfones, cellulose acetate, glass fibers, and inert metal fibers.

67. The method of claim 51, wherein the matrix is in a shape selected from the group consisting of squares, rings, discs, and cruciforms.

68. The method of claim 51, wherein the matrix is in the form of a disc.

69. The method of claim 51, wherein the matrix is coated with poly-D-lysine.

70. The method of claim 51, further comprising the step of isolating said undifferentiated hemopoietic stem cells or progenitor cells prior to step (b).

71. A method of transplanting undifferentiated hemopoietic stem cells or progenitor cells into a recipient, the method comprising the steps of:

- (a) expanding/maintaining the undifferentiated hemopoietic stem cells or progenitor cells by:
 - (i) obtaining undifferentiated hemopoietic stem cells or progenitor cells; and
 - (ii) culturing said undifferentiated hemopoietic stem cells or progenitor cells in a medium containing a stromal cell conditioned medium, said stromal cell conditioned medium being derived from a stationary phase plug-flow bioreactor in which a three dimensional stromal cell culture has been established on a substrate in the form of a sheet, said substrate including a non-woven fibrous matrix forming a physiologically acceptable three-dimensional network of fibers, thereby expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells.

72. The method of claim 71, wherein said undifferentiated hemopoietic stem cells or progenitor cells are cells isolated from a tissue selected from the group consisting of cord blood, mobilized peripheral blood and bone-marrow.

73. The method of claim 71, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture share common HLA antigens.

74. The method of claim 71, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from a single individual.

75. The method of claim 71, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from different individuals.

76. The method of claim 71, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from the same species.

77. The method of claim 71, wherein said undifferentiated hemopoietic stem cells or progenitor cells and stromal cells of said stromal cell culture are from different species.

78. The method of claim 71, wherein stromal cells of said stromal cell culture are grown to a density of at least 5×10^6 cells per a cubic centimeter of said substrate.

79. The method of claim 71, wherein stromal cells of said stromal cell culture are grown to a density of at least 10^7 cells per a cubic centimeter of said substrate.

80. The method of claim 71, wherein said fibers form a pore volume as a percentage of total volume of from 40 to 95 % and a pore size of from 10 microns to 100 microns.

81. The method of claim 71, wherein said matrix is made of fiber selected from the group consisting of flat, non-round, and hollow fibers and mixtures thereof, said fibers being of from 0.5 microns to 50 microns in diameter or width.

82. The method of claim 71, wherein said matrix is composed of ribbon formed fibers having a width of from 2 microns to 20 microns, and wherein the ratio of width to thickness of the fibers is at least 2:1.

83. The method of claim 71, wherein said matrix having a pore volume as a percentage of total volume of from 60 to 95%.

84. The method of claim 71, wherein the matrix has a height of 50-1000 μm .

85. The method of claim 71, wherein the material of the matrix is selected from the group consisting of polyesters, polyalkylenes, polyfluorochloroethylenes, polyvinyl chloride, polystyrene, polysulfones, cellulose acetate, glass fibers, and inert metal fibers.

86. The method of claim 71, wherein the matrix is in a shape selected from the group consisting of squares, rings, discs, and cruciforms.

87. The method of claim 71, wherein the matrix is in the form of a disc.

88. The method of claim 71, wherein the matrix is coated with poly-D-lysine.

89. A bioreactor plug comprising a container having an outlet and an inlet and containing therein a substrate in the form of a sheet, said substrate including a non-woven fibrous matrix forming a physiologically acceptable three-dimensional network of fibers, said substrate supporting at least 5×10^6 stromal cells per cubic centimeter of said substrate.

90. The bioreactor of claim 89, wherein said fibers form a pore volume as a percentage of total volume of from 40 to 95 % and a pore size of from 10 microns to 100 microns.

91. The bioreactor of claim 89, wherein said matrix is made of fiber selected from the group consisting of flat, non-round, and hollow fibers and mixtures thereof, said fibers being of from 0.5 microns to 50 microns in diameter or width.

92. The bioreactor of claim 89, wherein said matrix is composed of ribbon formed fibers having a width of from 2 microns to 20 microns, and wherein the ratio of width to thickness of the fibers is at least 2:1.

93. The bioreactor of claim 89, wherein said matrix having a pore volume as a percentage of total volume of from 60 to 95%.

94. The bioreactor of claim 89, wherein the matrix has a height of 50-1000 μm .

95. The bioreactor of claim 89, wherein the material of the matrix is selected from the group consisting of polyesters, polyalkylenes, polyfluorochloroethylenes, polyvinyl chloride, polystyrene, polysulfones, cellulose acetate, glass fibers, and inert metal fibers.

96. The bioreactor of claim 89, wherein the matrix is in a shape selected from the group consisting of squares, rings, discs, and cruciforms.

97. The bioreactor of claim 89, wherein the matrix is in the form of a disc.

98. The bioreactor of claim 89, wherein the matrix is coated with poly-D-lysine.

99. A plug-flow bioreactor comprising the bioreactor plug of claim 89.

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